This effect of insulin on macrophages could also be blocked by a serum from a diabetic patient with high insulin resistance and capable of reducing about 80% the uptake of ^{125}I-insulin by macrophages. Several other substances could also inhibit the stimulatory effect of insulin on macrophages: rabbit anti-macrophage serum (1:20 dilution), trypsin (1 mg/ml), phospholipase A and C (100 µg/ml), deoxyglicose (50 µg/ml), theophylline (10^{-3} M) (table).

Discussion. Previous reports[13,14] from our laboratory demonstrated that immunological phagocytosis by mouse peritoneal macrophages can be influenced by drugs which modulate the intracellular levels of cyclic nucleotides (cAMP and cGMP). It was shown that agents known to raise the intracellular levels of cAMP are inhibitors of phagocytosis, while phagocytosis was enhanced by cholinergic drugs which raise the intracellular levels of cGMP. In this paper we demonstrated that in physiological concentrations, insulin enhanced, in vitro, the immunological phagocytosis of SRBC sensitized with rabbit IgG by normal mouse peritoneal macrophages. With higher concentrations, there were no significant changes in the ingestion index. According to our results, insulin seems to stimulate macrophage phagocytosis as a cholinomimetic agonist by increasing the intracellular levels of cGMP. Insulin has been reported to have a physiological effect on macrophage functions, but the results obtained are somewhat contradictory. Rhodes[15], for instance, showed that the macrophage-Fc expression is inhibited by insulin (10 µg/ml or 50 µg/ml), dibutyryl AMP and methylxanthine, but is as augmented by the dibutyryl cGMP. According to Muschel et al.[5], insulin at concentration of 60 ng/ml produced a marked depression in Fc-mediated phagocytosis by mouse peritoneal macrophages, and that its effect was reversed by cAMP, isoproterenol and cholera toxin.

The conclusions that insulin inhibits macrophage-Fc expression[16] and that cAMP enhances and insulin inhibits Fc-mediated ingestion by peritoneal macrophages, are in conflict with our results. We have no explanation for these discrepancies, but at least 2 main technical variants could well be invoked to elucidate them: a) different concentrations of insulin used for attaining maximal effects; b) different methods for evaluating the results obtained. The evaluation of experimental results by taking into account only the percentage of cells engaged in phagocytosis is fallacious. It is also necessary to determine the ingestion index, as can easily be seen from our data.

1 Acknowledgments. This investigation was supported by a grant from the National Council for Cientific and Tecnological Development (CNPq), Rio de Janeiro, Brazil.
2 J.R. Gavin, J. Roth, P. Jen and P. Freichet, Proc. natl Acad. Sci. *69*, 747 (1972).
3 R.H. Schwartz, R. Bianco, B.S. Handwerger and C.R. Kahn, Proc. natl Acad. Sci. *72*, 474 (1975).
4 J.R. Gavin, D.N. Buell and J. Roth, Science *178*, 168 (1972).
5 R.S. Bar, C.R. Kahn and H.S. Koren, Nature *265*, 632 (1977).
6 R.J. Muschel, N. Rosen, O.M. Rosen and B.R. Bloom, J. Immun. *119*, 1813 (1977).
7 T.B. Strom, R.A. Bear and C.B. Carpenter, Science *187*, 1206 (1975).
8 J.H. Helderman and T.B. Strom, J. clin. Invest. *59*, 338 (1977).
9 J.H. Helderman and T.B. Strom, Science *274*, 62 (1978).
10 N.B. Goldberg, M.K. Haddox, R. Stessen, C. Lopez and J.W. Hadden, in: Cyclic AMP, immune response and tumor growth. Ed. L.M. Lichtenstein, W.E. Braun and C.W. Parker. Springer, New York 1977.
11 G. Iliano, P.E.G. Tell, M.I. Siegel and P. Cuatrecasas, Proc. natl Acad. Sci. *70*, 2443 (1973).
12 C. Bianco, F.M. Griffin and S.C. Silverstein, J. exp. Med. *141*, 1278 (1975).
13 A. Oliveira Lima, M.Q. Javierre, W. Dias da Silva and D. Sette Camara, Experientia *30*, 945 (1974).
14 M.Q. Javierre, A. Oliveira Lima, L.V. Pinto and W. Dias da Silva, Revta bras. Pesq. Med. Biol. *8*, 119 (1975).
15 J. Rhodes, Nature *257*, 597 (1975).

Effects of castration, estradiol and testosterone on tubulin levels of the medial basal hypothalamus and the adenohypophysis of the rat[1]

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Summary. Tubulin levels of the medial basal hypothalamus (MBH) were greater in male than in female rats. Orchidectomy brought about a decrease of MBH tubulin concentration, whereas testosterone injection augmented it in the MBH and adenohypophysis. Estradiol administration augmented MBH tubulin and protein concentration.

The biochemical and pharmacological properties of the protein constituting microtubules (tubulin) have been well characterized. Its binding to the antimitotic drug colchicine exhibits a specific and stoichiometric affinity which has provided an assay for estimating tubulin concentration in a number of tissues, including the brain[3]. In this tissue tubulin comprises 15–40% of the total soluble protein and its high concentration has been one of the most significant factors to indicate that microtubules may play an important functional role in nervous tissue. Mainly based upon indirect observations by using agents (e.g., colchicine or vinblastine) which disrupts microtubules, these organelles have been implicated in both axoplasmic transport and neurosecretion[4]. Very few observations have been published concerning changes of colchicine-binding activity of the brain as a function of the neuroendocrine status of the animal. Hypothalamic tubulin was found to be affected by catecholamine transmitter through alpha- and beta-adrenergic receptors, the latter involving the pineal gland[5], and presumably melatonin secretion[6]. Experimental manipulations known to alter the neuroendocrine apparatus, such as continuous exposure to light or superior cervical sympathectomy, also resulted in modification of colchicine-binding activity of the hypothalamus[5]. The present experiments were undertaken to examine the effects of estradiol and testosterone on tubulin levels of the medial basal hypothalamus (MBH) and the adenohypophysis (AH) of castrated rats.

Material and methods. Adult Wistar rats (180–220 g) were kept under controlled lighting from 07.00 to 21.00 h daily

Effect of testosterone and estradiol treatment on tubulin levels of medial basal hypothalamus (MBH) and adenohypophysis (AH) of castrated rats

Treatment	MBH Tubulin concentration (nmoles · mg protein^{-1})	MBH Soluble protein concentration (mg · g tissue^{-1})	AH Tubulin concentration (nmoles · mg protein^{-1})	AH Soluble protein concentration (mg · g tissue^{-1})
Male rats				
Vehicle	1.39±0.07 (26)	4.82±0.40 (12)	1.16±0.12 (26)	7.05±0.2 (12)
Testosterone	2.15±0.09 (21)**	4.74±0.57 (13)	1.94±0.12 (24)**	6.02±0.3 (12)*
Female rats				
Vehicle	1.45±0.05 (31)	4.01±0.18 (15)	0.84±0.05 (32)	5.22±0.25 (15)
Estradiol	1.41±0.05 (30)	5.18±0.31 (14)**	0.77±0.05 (28)	5.57±0.19 (15)

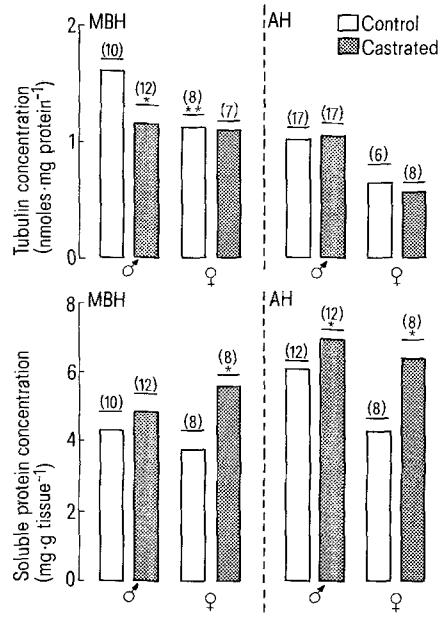
Results are expressed as mean±SEM (n). Asterisks represent significant differences from vehicle-injected controls: * $p<0.05$; ** $p<0.01$. Student's t-test.

and were given access to chow and water ad libitum. For testosterone experiments, males castrated 72 h earlier were treated for 3 days with 2 daily s.c. injections (at 09.00 and 17.00 h) of 400 µg of testosterone dissolved in 0.2 ml of saline: ethanol (1:1). An additional injection of 400 µg of testosterone was given on the 4th day, 3 h before sacrifice; controls received the vehicle alone. For estradiol experiments, female rats spayed 3 weeks earlier were injected on 2 consecutive days with daily s.c. injections of 0.5 and 50 µg of the hormone, respectively; the animals were killed in the morning of the 3rd day. Controls received s.c. injections of the vehicle (0.2 ml of saline: ethanol, 3:1). The animals were killed by decapitation, the brains were quickly removed, and the MBH was dissected out as described elsewhere[7]. The AH and MBH were weighed and homogenized in 0.067 M Na phosphate buffer pH 6.8, containing 0.1 M KCl and 0.1 mM GTP. Colchicine-binding was assayed as described by Sherline et al.[8], by equilibrating the homogenates with ^{3}H-colchicine (0.02 mM) during 90 min at 37 °C after which the remaining free colchicine was removed by adsorption onto activated charcoal. Following precipitation of charcoal by centrifugation for 15 min at 600×g, aliquots of the supernatants were taken for ^{3}H-radioactivity measurement by liquid scintillation spectrometry, and for determining the protein concentration by the Lowry et al. procedure[9]. The results were expressed as nmoles of tubulin per mg of soluble protein, by assuming that at saturation 1 mole of colchicine was bound per mole of tubulin[10].

Results. Tubulin concentration of MBH of female rats was significantly lower than in male rats (figure). Castration of male, but not of female rats, decreased MBH tubulin levels by 40%. A significant increase of soluble protein concentration was detected in the AH of castrated males and in the MBH and AH of castrated females (figure).

The administration of testosterone to castrated males brought about a significant, 55%-increase of colchicine-binding activity of the MBH without affecting soluble protein concentration (table). Testosterone also augmented tubulin levels of the AH by 67% and decreased soluble protein concentration by 15%. Estradiol injection to oophorectomized rats did not change MBH tubulin levels, expressed per mg of protein, whereas it increased soluble protein concentration by 29%; hence the amounts of tubulin per mg of tissue augmented by 23% (9.47±0.99 and 11.68±1.08 nmoles·mg of tissue^{-1}, in vehicle- and estradiol-treated rats respectively). The addition of 10^{-6} or 10^{-8} M testosterone or estradiol to hypothalamic or hypophysial homogenates did not affect colchicine-binding to tubulin (results not shown).

Discussion. The importance of the MBH in gonadotrophic control is now well established. The hypophysiotrophic



Effect of castration on tubulin levels of the MBH and AH of rats. Data are presented as mean±SEM (n). *Significantly different from its respective control: $p<0.05$; Student-t-test. **Significantly different from its respective control male: $p<0.05$.

area, defined as the region of the 3rd ventricle that will maintain a transplanted AH in an active secretory state, coincides with the MBH, and considerable anatomical and electrophysiological data indicate that this region contains neurons that project axons to the median eminence having access to the portal system[11]. The foregoing results indicate that MBH tubulin levels are lower in female than in male rats, and that orchidectomy brought about a decrease of MBH tubulin levels, which was counteracted by testosterone administration. In female rats, estradiol increased marginally MBH tubulin levels. Since, in intact female rats killed irrespective of the stage of the estrous cycle, the variance observed was not significantly greater than in any other group, it can be provisionally concluded that MBH and AH tubulin levels are independent of estrous cycle. Additional experiments are needed further to substantiate this conclusion.

While the physiological significance of microtubules in neuroendocrine tissues remains uncertain, it seem feasible that they participate in 2 processes of major functional importance, such as axoplasmic transport and hormone secretion. Colchicine and vinblastine inhibit the movement

of neurosecretory granules from the hypothalamic nuclei to the median eminence and to the neurohypophysis, and block the release of vasopressin from the posterior lobe[12,13]. Neurons from the preoptic area produces LHRH which is transported to the MBH by an axonal route for storage and/or release into the hypophysial portal veins[14]. Ultrastructural studies have lent support to the participation of microtubules in hormone secretion from the AH[15]. Therefore it seems feasible that changes in tubulin levels of MBH and AH following castration and hormone replacement may be associated with modification of transport and/or secretions of various materials of neuroendocrine significance.

1 This study was supported in part by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina (CONICET) (grant No. 6638), and the Programa Latinoamericano de Investigación en Reproducción Humana (PLAMIRH 2.3.1.75 R).

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3 M.L. Shelanski, in: Research Methods in Neurochemistry, vol II, p. 281. Ed. N. Marks and R. Rodnight. Plenum Press, New York 1974.
4 L. Wilson, Fedn Proc. *33*, 151 (1974).
5 D.P. Cardinali, F. Freire, C.A. Nagle and J.M. Rosner, Neuroendocrinology *19*, 44 (1975).
6 D.P. Cardinali and F. Freire, Molec cell. Endocr. *2*, 317 (1975).
7 D.P. Cardinali and E. Gómez, J. Endocr. *73*, 181 (1977).
8 P. Sherline, C. Bodwin and D.M. Kipnis, Analyt Biochem. *62*, 400 (1974).
9 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
10 J.A. Snyder and J.Mc Intosh, A. Rev. Biochem. *45*, 721 (1976).
11 J. Szentágothai, B. Flerkó, B. Mess and B. Halász, in: Hypothalamic control of the Anterior Pituitary. Akadémiai Kiadó. Budapest 1968.
12 A. Norström, Ann. N.Y. Acad. Sci. *248*, 46 (1975).
13 E.L.R. Echandía, J.C. Cavichia and E.M. Rodríguez, J. Endocr. *73*, 197 (1977).
14 S.P. Kalra, P.S. Kalra and E.O. Mitchell, Endocrinology *100*, 201 (1977).
15 F. Labrié, M. Gauthier, G. Pelletier, P. Borgeat, A. Lemay and J.J. Gouge, Endocrinology *93*, 903 (1973).

Effect of α- and β-ecdysone on DNA synthesis in *Aeshna cyanea* (Insecta, Odonata) midgut

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Summary. In *Aeshna cyanea* larvae, α- and β-ecdysone stimulate DNA synthesis in the midgut regenerative cells. In last larval instar, the number of cells obtained after the imaginal epithelium genesis is greater after α- than after β-ecdysone supply. Such a result should be compared with the imaginal epithelium differentiation which occurs earlier after β-ecdysone injection.

It is well known that insect post-embryonic development is controlled by hormones. Numerous are the morphological and biochemical events attribuable to the action of ecdysones. Recent studies seem to indicate clearly the role of ecdysones in the stimulation of RNA synthesis[1]. On the other hand, the published data concerning the control of DNA synthesis do not appear to agree.

The following questions have not yet been answered completely: a) Is DNA synthesis under the control of ecdysones? Although some authors have shown a stimulatory in vivo or in vitro effect of α-[2-6], β-ecdysone[3,7,8] or inokosterone[9,10], the results often differ according to animal species, depending upon the developmental stage of the target organ[11] and the ecdysone level[6,12-15]. Moreover, Bullière and Bullière[10] have recently suggested that the inhibitory or triggering effect of the moulting hormone is function of the proliferative or differentiative nature of the epidermal divisions. b) Is α-ecdysone a true hormone having a specific role particulary on DNA synthesis prior to its conversion to β-ecdysone, as suggested by Oberlander[6]? Do α- and β-ecdysone effect DNA synthesis in the same way?

To answer these questions we have chosen as a model the midgut of a dragonfly, *Aeshna cyanea,* for several reasons: a) By its endodermal origin, the insect midgut is quite different from the organs usually studied in the control of DNA synthesis. b) This target organ contains principally a single cell type, the functional cells which, throughout the insect's life, are replaced from small embryonic cells, the regenerative cells, grouped in nidi, near the basement membrane. c) The last larval instar midgut of *A. cyanea* is characterized by the genesis of 2 tissues: the reticulate tissue and the imaginal epithelium which both result from the differentiation of the regenerative cells[16]. The first event of the midgut in this instar is therefore a proliferating activity of these cells. d) In controls, imaginal epithelium genesis occurs when the sclerification of the tarsal claws takes place. This tegument response, easily observable after α- or β-ecdysone injection led us to a first study[17] in which the experimental animals were killed some h after this event. At this time, we observed that there was always an increase in the number of regenerative cells. Furthermore, by means of an autoradiographic study we were able to demonstrate that β-ecdysone accounts for this increase in stimulating DNA synthesis.

It was also noted that identical amounts of α- and β-ecdysone give different results. Thus, as a general rule, the genesis of the imaginal epithelium occurs later when α- instead of β-ecdysone is supplied. In the same way, it may be asked whether α- and β-ecdysone stimulate DNA synthesis in a similar way.

Material and methods. We used antepenultimate, penultimate and last instar larvae of *Aeshna cyanea* which were injected on the 5th day of the instar with a 10% alcoholic solution of α- (Simes) or β-ecdysone (Sigma). Indices of cell proliferation were the number of mitosis (meta- and anaphase figures) and the number of regenerative cells per transversal section. 20 serial sections were studied to determine the number of mitotic figures per section.

Results. Figure 1 records the values of the mitotic index during the 10 days following the 20 µg/g α- or β-ecdysone supply to 55 last larval instar. These results demonstrate clearly that after α- or β-ecdysone injection there is always an increase of the mitotic index. This graph also shows that, at first, α- and β-ecdysone stimulate DNA synthesis in the same way. The time course of mitosis and the number of